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# New Approaches for Improving Engraftment after Cord Blood Transplantation

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Use of unrelated umbilical cord blood cells (UCB) as an alternative source of hematopoietic cell transplantation (HCT) has been widely used mainly for patients lacking an HLA-matched donor. There are many advantages for using CB cells over bone marrow or mobilized peripheral blood (MBP) from volunteer donors, such as rapid availability, absence of risk for the donor, or decreased incidence of acute graft-versus-host disease (aGVHD). However, a significant clinical problem is delayed engraftment, which is directly correlated with the number of hematopoietic stem cells (HSCs) in a CB unit. The understanding of methods to improve collection, expansion, and homing of CB cells, the identification of prognostic factors associated with engraftment that can be easily modified (eg, strategies for donor choice), and development of new approaches including use of multiple donors, cotransplantation with accessory cells are of crucial importance to circumvent the problem of delayed engraftment after UCB transplantation. Those approaches may greatly increase the quality and availability of CB for transplantation.

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**KEY WORDS:** Umbilical cord blood transplantation, Engraftment, Expansion, Homing, Double cord blood transplantation

## INTRODUCTION

Umbilical cord blood (UCB) transplantation has extended the availability of allogeneic hematopoietic cell transplantation (HCT) to patients who would otherwise not be eligible for this curative approach. Progress in the field of UCB transplantation parallels the expanding interest in establishing and developing CB banks worldwide. Today, more than 450,000 CB grafts are available in more than 50 CB banks, and it is estimated that more than 20,000 UCB transplantations have been performed worldwide. Currently more than 2000 transplants are being performed yearly around the world (World Marrow Donor Association [WMDA] oral communication, March 2009). In comparison with other sources of allogeneic HCT, UCB offers substantial logistic and clinical advantages [1]. However, the main problem with using UCB for transplantation is the relatively low number of hematopoietic progenitor cells (HPC) and hematopoietic stem cells (HSC) in UCB compared with bone marrow (BM) or mobilized peripheral blood (MPB) grafts, which translates into increased risk of graft failure, delayed hematopoietic engraftment [2-6], and delayed immune reconstitution [7,8]. The cumulative incidence of nonengraftment after UCB transplantation varies from 10% to 20% and the median time to neutrophil recovery varies from 22 to 27 days. Death frequently occurs during aplasia because of opportunistic infections without any sign of engraftment. However, despite this acute problem, long-term outcomes of survival and disease-free survival (DFS) after UCB transplantation are comparable to other sources of HCT because the incidence of graft-versus-host disease (GVHD) is reduced [2-6].

A number of biological factors, including the number and/or immaturity of HSC or HPC, limited number of accessory cells for engraftment (lymphocytes subsets or mesenchymal cells), and homing capacity, may be involved as mechanisms to explain this delayed engraftment.

Many approaches have been investigated to enhance collection of HSC and HPC in CB units (CBUs) and to improve homing of CB cells. Examples include blocking or stimulating specific peptides or injecting CB cells directly into the BM [9]. Other new approaches for in vivo or ex vivo amplification of CB cells have been developed [10,11]. Importantly, modifiable factors related to donor CBU choice (such as cell dose and degree of HLA incompatibility), and transplantation procedures (such as conditioning regimen

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**Table 1. Experimental and Clinical Approaches to Increase Number of Cord Blood Cells and Improve Engraftment after UCB Transplantation**

- Increase number of cells at cord blood collection
  - Banking cord blood units with greater volume and high number of CD34+ cells
  - Perfusing the placental vessels after draining the blood from the cord
- Enhance homing of cord blood cells
  - Inhibiting the enzymatic activity of CD26/Dipeptidylpeptidase IV (DPPIV)
  - In vivo direct injection of cord blood cells into the iliac crest (Phase II clinical trials)
- In vitro and in vivo expansion of cord blood cells
  - Using of SDF-1/CXCL12 associated to Diprotin A and/or other cytokines
  - Using Notch-ligand Delta 1 (Phase II clinical trials)
  - Using copper chelator tetraethylenepentamine (TEPA) (Phase II clinical trials)
- Identification of modifiable prognostic factors for engraftment
  - Choosing the "best" cord blood unit based on cell dose, HLA, diagnosis, screening for antibodies against HLA, quality of cord blood units (Table 2)
  - Modifying the conditioning regimen and GVHD prophylaxis
- Increase number of cells at infusion
  - Using double cord blood transplantation (on going prospective and observational studies)
  - Using third party mobilized T cell-depleted haploidentical cells (Phase II trials)
- Decreasing toxicity and shorten time of aplasia
  - Using RIC (on going prospective and observational studies)
- Coinfusion of cord blood cells with accessory cells
  - Using multipotent mesenchymal stromal cells (Phase I/ II trials)

GVHD indicates graft-versus-host disease; UBC, umbilical cord blood; RIC, reduced intensity conditioning regimen.

and GVHD prophylaxis) have been identified to guide clinicians [12-22].

Other approaches aimed at decreasing toxicity and shortening the time to engraftment after UCB transplantation have shown encouraging results such as the use of double unit CB transplants [23,24], the use of reduced-intensity conditioning (RIC) regimens [25,26], coinfusion with a haploidentical T cell-depleted graft [27,28], or coinfusion with multipotent mesenchymal stromal cells (MSCs) [29].

Table 1 lists experimental and clinical approaches to circumvent the engraftment problem after UCBT.

## Enhancing Collection, Homing, and Expansion of CB Cells

Because of the limiting numbers of HSCs and HPCs in banked CB, the means to: (1) enhance collection of CB cells [30], (2) enhance the homing and engraftment of HSCs /HPCs [31,32], and/or (3) enhance the ex vivo or in vivo expansion of these cells could greatly enhance the quality and usefulness of CB cells for transplantation.

The CB cells collected for use in the first CB transplants were composed of cells drained from the cord, followed by retrieval of cells left in the placental blood vessels to increase the numbers of cells for transplantation [12]. It is possible to substantially enhance the numbers of HPC collected by perfusing the placental vessels after draining the blood from the cord [30], but the practicality of this method for banking remains to be evaluated. If perfusion of the placenta after collection of blood from the cord is untaken, it would need to be done in selected collection centers with well-trained personnel.

There have been a number of efforts to enhance the homing and engraftment capability of HSCs and HPCs. One such means is to inhibit the enzymatic activity of CD26/Dipeptidylpeptidase IV (DPPIV) with small peptides [31,32]. CD26/DPPIV truncates and inactivates the chemokine stromal cell derived factor-1 (SDF-1/CXCL12) that binds a receptor, CXCR4. The SDF-1/CXCL12-CXCR4 axis is known to be important in the in vitro chemotaxis (directed cell movement) and in vivo homing of mouse and human HSCs. Not only is the truncated SDF-1/CXCL12 inactive, but it also blocks the actions of the full-length active form of SDF-1/CXCL12. This finding suggested that a means to enhance SDF-1/CXCL12 activity by preventing its truncation by CD26/DPPIV might enhance the homing and engraftment of HSCs and HPCs. Using small peptide inhibitors of CD26/DPPIV such as Diprotin A or Val-Pyr, it was possible to enhance the homing and engrafting capability of mouse BM HSCs into lethally irradiated mice, and human CB HSCs into sublethally irradiated NOD/SCID mice [32]. SDF-1/CXCL12 also acts as a survival factor for HSCs/HPCs, and most recently, SDF-1/CXCL12 was found to enhance the ex vivo expansion of CB HPCs induced by the combination of stem cell factor, Flt3-ligand, and thrombopoietin [30]. The addition of Diprotin A to SDF-1/CXCL12 further enhanced ex vivo output of HPCs (H.E. Broxmeyer, unpublished). In addition to SDF-1/CXCL12 and a number of other chemokines, there are other cytokines that have these putative CD26/DPPIV truncation sites. Using Diprotin A or $CD26^{-/-}$ cells, it was possible to enhance the activity of selected members of the colony stimulating factor (CSF) family in vitro. Moreover, it was determined that HPCs recovered much more rapidly and at a higher level in $CD26^{-/-}$ mice compared to control mice after cytotoxic stress from low and higher, but not lethal, doses of irradiation and from the chemotherapeutic drugs, 5-flurouracil, and cyclophosphamide (Cy; H.E. Broxmeyer, J. Hoggatt, S. Cooper, G. Hangoc, L.M. Pelus, and T.B. Campbell, manuscript in preparation). It is conceivable that pretreatment of donor cells, and/or the recipients may enhance engraftment of limiting numbers of HSC/HPC, such as are present in CB collections.

Other mechanisms are under investigation with the aim to improve ex vivo expansion of cord blood cells. Phase I/II clinical trials have started to evaluate safety and toxicity of infusing Notch-ligand Delta 1 or copper chelator tetraethylenepentamine (TEPA; StemEx) to induce ex vivo expansion of CB progenitors in patients with hematologic malignancies [10,11]. Interestingly, Notch-ligand Delta 1 has also

been shown to have an effect on early T cell expansion and differentiation [33].

Future efforts to expand HSC/HPC ex vivo and in vivo, and to enhance the homing and engrafting capabilities of CB cells will likely make use of more in depth information on intracellular signaling molecules and their networks involved in HSC and HPC functions, including self-renewal, proliferative, survival, differentiation, and migration [34]. Further information on the BM microenvironment and how HSC/HPC interact with this microenvironment will permit the development of more effective ways to achieve engraftment.

## Enhancing Homing Capacity with Direct Intrabone Marrow Injection of CB Cells

The concept of enhancing homing capacity of CB cells through the direct injection of CB cells into the BM environment has led some investigators to apply this approach clinically. In mice, it has been suggested that intrabone infusion of $CD34^+$ CB cells confers an engraftment advantage 15 times greater than after intravenous infusion, because cell loss during circulation before homing is circumvented [35]. Recently, a phase I/II study was performed to establish the safety and efficacy of a new administration route (intrabone) for CB cells, measured by the donor-derived neutrophil and platelet engraftment. Thirty-two patients had leukemias, 14 with advanced disease. HLA-matching was 5/6, 4/6, and 3/6 for 9, 22, and 1 patient, respectively. Most of the patients received a myeloablative (MA) conditioning regimen associated with antithymocyte globulin (ATG) and only 2 patients recived an RIC regimen prior to UCB transplantation. CB cells were concentrated in 4 5-mL syringes, and were infused in the superior-posterior iliac crest under rapid general anaesthesia. Median transplanted cell dose was $2.6 \times 10^7$/kg. No complications occurred during or after the intrabone infusion of cells. Median time to recovery of neutrophils was 23 days (range: 14-44) and median time to recovery of platelets was 36 days (range: 16-64). All patients were fully chimeric from 30 days after transplantation to the last follow-up visit, suggesting early complete donor engraftment. No patient developed grade III-IV acute graft-versus-host disease (aGVHD). More recently, in a preliminary matched pair analysis comparing patients transplanted with CB injected intravenously (IVCB) versus CB injected directly into the BM (IBCB) of the iliac crest, IBCB patients (n = 50) were matched with 88 IVCB recipients. Cumulative incidence (CI) of neutrophil recovery was 70% ± 5% in IVCB recipients versus 80% ± 6% in the IBCB group ($P = .27$). However, patients receiving IBCB had a higher CI of platelet recovery at day 60 (82% ± 5%) compared to the IVCB group (40% ± 5%; $P < .0001$). Strikingly, the incidence of aGVHD grade II-IV was 12% in the IBCB group compared to 38% in the IVCB group ($P = .0001$) and the incidence of grade III-IV aGVHD was 2% compared to 18% ($P < .001$), respectively. Overall survival (OS) at 1 year was 67% ± 7% compared to 43% ± 5% ($P = .07$), respectively. In summary, injection of CB cells into the BM appears to significantly reduce the problem of delayed platelet recovery observed after IVCB. The reduced incidence and severity of aGVHD observed in IBCB patients is intriguing and promising [9]. This procedure has been adopted in some European transplant centers in phase I/II clinical trials, in which that a collected nucleated cell dose is found cell dose between 1.5 and $2.5 \times 10^7$/kg with the aim to avoid performing double cord blood transplants.

## Risk Factors That Affect Engraftment: CB Graft and Transplantation-Related Factors

### *Interactions of cell dose, HLA, and diagnosis*

As cell dose and HLA disparities are important and independent prognostic factors, it has been suggested that both interact mutually on engraftment and other outcomes. Thus, a higher cell dose in the graft should partially overcome the negative impact of HLA mismatch for each level of HLA disparity. It has been speculated that for each HLA disparity, the nucleated cell dose should be increased by $1.5 \times 10^7$/kg. However, as the hypothesis has not been fully tested and the recommendation at the present time is unvalidated, this approach has yet to be adopted. Using this algorithm many patients will not find a suitable single CBU and will need a double CB transplant. The Eurocord group and others have made some recommendations regarding criteria for donor choice. A strategy for CBU choice based on cell dose, HLA match, and diagnosis is listed in Table 2 [36].

The following considerations should be taken into account when choosing a CBU to improve engraftment:

1. Cell content marker: the $CD34^+$ cell content at the time of freezing is considered a better marker for HSC and HPC than either the total nucleated cell (TNC) at the time of freezing or the $CD34^+$ cell content after thawing. Colony-forming units granulocyte-macrophage (CFU-GM) in the CB graft represents clonogenic potential. However, because of logistic, technical, and economic issues, the use of this cell marker is difficult to apply routinely as a surrogate marker for CBU choice.
2. HLA allelic matching: high-resolution typing for HLA-A, -B, and -DRB1 was associated with decreased incidence of aGVHD and a trend toward improved survival, but had no impact on neutrophil or platelet engraftment after UCB transplantation in children [20]. To determine the real value of allele

**Table 2. Considerations for Cord Blood Unit Choice**

1) At selection, diagnosis and presence of patient HLA antibodies against the HLA antigens [37] of the cord blood unit should be considered. HLA compatibility appears to be more important for patients with nonmalignant disorders than for those with malignant disorders [1].
2) If the below criteria for a single UCB transplantation is not achieved, a double cord blood transplantation should be considered in prospective trials. HLA definition: based on HLA antigenic for -A and -B and allelic typing for HLA-DRB1. Avoid cord blood units with 3 or 4 HLA disparities.

Recommendations for HLA and cell dose (speculative)

1) Cord blood unit with 6/6 or 5/6 HLA match. HLA-A or HLA-B mismatches are preferable to DRB1 mismatches. HLA-DRB1 mismatch could probably lead to high graft-versus-leukemia (GVL) effect in patients transplanted in nonremission (based on Eurocord unpublished and preliminary data).

Malignant disorders:

Nucleated cell dose: at freezing, minimum cell dose 2.5 to 3.0 $\times$ $10^7$/kg after thawing, minimum 2.0 to 2.5 $\times$ $10^7$/kg*
(this recommendation is partially based on Eurocord, New York Placental Blood Center and CIBMTR data) [6,13,15].

*If the nucleated cell dose infused is <1.0 $\times$ $10^7$/kg, an immediate second transplant should be considered because the early mortality associated to this cell dose is approximately 70%. If the nucleated cell dose infused is between 1.0 to 2.0 $\times$ $10^7$/kg, we recommend that the number of $CD34^+$ cells and CFU-GM should be taken into consideration to predict the probability of neutrophil recovery and to consider a second transplant. If both cell doses are lower than recommended, a bone marrow aspirate and chimerism analysis should be performed between day +20 and day +28 to confirm the absence of engraftment and to indicate a second transplant.

$CD34^+$ cell dose: at freezing or after thawing, approximately 1.2 to 1.7 $\times$ $10^5$/kg (this recommendation is based on data published [13,16,17]
Colony forming units assay: when available, the cord blood bank should inform the value and technique of CFU-GM counting.

Nonmalignant disorders: same total and $CD34^+$ cell dose requested, but HLA match should always be selected and avoid DRB1 mismatching.
Cord blood unit with 4/6 HLA match (this recommendation is not proved by retrospective studies, but based on transplant algorithm for cord blood unit selection and unpublished Eurocord data) HLA-A or HLA-B mismatches are better than HLA-DRB1 mismatches. HLA-DRB1 mismatch could probably lead to high GVL effect in advanced phase of the diseases (based on Eurocord unpublished and preliminary data).

Malignant disorders

Nucleated cell dose: at freezing, minimum cell dose 3.5 $\times$ $10^7$/kg after thawing, minimum 3.0 $\times$ $10^7$/kg*
$CD34^+$ cell dose: at freezing or after thawing, approximately >1.7 $\times$ $10^5$/kg
Colony forming units assay: when available, the cord blood bank should provide the CFU-GM count and the method used to determine this result.

Nonmalignant disorders (based on unpublished Eurocord study)
Nucleated cell dose: at freezing, minimum cell dose 4 to 5 $\times$ $10^7$/kg after thawing, minimum 3.5 $\times$ $10^7$/kg*
$CD34^+$ cell dose: no available data, but should be higher than 2 to 2.5 $\times$ $10^5$/kg
Colony forming units assay: when available, the cord blood bank should provide the CFU-GM count and the method used to determine this result.

2) CB units with 3/6 HLA match: should be avoided, but in extremely severe cases for patients with malignant disorders should be considered with high nucleated cell dose. Not recommended for patients with nonmalignant disorders.

Other considerations:

If a number of cord blood units are available that fit the above criteria, the following should to be taken into consideration:
1) Accredited Cord blood bank and location
2) ABO compatibility
3) Allele HLA typing of HLA-A and -B

UCB indicates umbilical cord blood; CIBMTR, Center for International Blood and Marrow Transplant Research; CFU-GM, Colony-forming units granulocyte-macrophage.

typing in UCB transplantation, thousands of patient-donor pairs will be needed to reach statistical significance.

3. Patients' pretransplant anti-HLA antibodies: preformed anti-HLA antibodies also seem to have an impact on neutrophil and platelet engraftment after UCB transplantation. Because most UCB transplants are HLA mismatched, the presence of anti-HLA antibodies in the patient against HLA epitopes of the CBU should be investigated prior to transplant [37].
4. Unrelated donor CB banks—economic and quality aspects: economic concerns (such as costs of the price unit in double CB transplantation), the distance of CB banks, and, more importantly, the quality of the CBU are considerations when selecting a CBU. However, very few studies have analyzed the impact of CB banking procedures (such as prefreezing manipulation, volume reduction, viability of CB cells after thawing, methods of cryopreservation and thawing, etc.) on engraftment and other outcomes after UCB transplantation.

### *Transplantation related factors: conditioning regimen and GVHD prophylaxis*

Factors related to the technique of transplantation, such as conditioning regimen and GVHD prophylaxis may also be associated with more rapid engraftment.

In a recent Eurocord study, the use of fludarabine (Flu) in MA conditioning regimens was associated with improved neutrophil and platelet recovery in adult UCB transplantation recipients receiving a lower TNC dose [21]. In this study, the role of antithymocyte globulin (ATG)/antilymphocyte globulin (ALG) could not be evaluated as it was used in almost all UCB transplantation performed. Use of Flu in the preparative regimen has also been associated with improved engraftment independent of cell dose and HLA in UCB transplantation for patients with Fanconi anemia (FA) [38]. Conversely, the use of methotrexate (MTX) containing regimens for GVHD prophylaxis has been associated with delayed engraftment and increased risk of graft failure in patients with hemoglobinopathies transplanted with an HLA identical sibling CBU [22]. However, its use elsewhere in

UCB transplantation is not established. In Europe and the United States, the most common regimen is calcineurin inhibitor-based GVHD prophylaxis alone or in combination with steroids or mycophenolate mofetil (MMF). However, Japanese transplant centers have shown interesting results with calcineurin inhibitors in combination with low-dose MTX [39,40]. Only prospective studies may establish the role of MTX in GVHD prophylaxis for UCB transplantation.

## New Approaches to Improve Engraftment and Decrease Early Transplantation Mortality

Because cell dose is considered to be a critical determinant of outcomes in UCBT, the Minneapolis group has demonstrated that transplantation of 2 partially HLA matched cord units may overcome the problem of cell dose and make the transplantation of heavier adult patients feasible. This strategy has led to an increased number of adult patients receiving unrelated cord blood transplantation. Results with double cord blood transplantation support the safety of the procedure [23,24]. Chimerism data from these studies reveal that typically only 1 cord blood engrafts. Despite the fact that double CB transplant recipients are heavier than patients receiving a single unit, cumulative incidence of neutrophil recovery does not differ statistically between the 2 groups. This observation suggests a "booster" effect from the nonengrafting unit. Recent data from the Minnesota group suggests that double UCB transplantation is associated with a higher incidence of aGVHD, when recipients of single (n = 210) versus double (n = 169) UCB transplantation have been compared, but without an increase in nonrelapse mortality (NRM). Interestingly, analysis of 177 patients with acute leukemia wherein 47% of patients were given single CBUs and 53% given 2 partially HLA-matched units, relapse was significantly lower for early stage (CR1-2) patients who received 2 UCB units, suggesting a higher graft-versus-leukemia (GVL) effect. Leukemia-free survival (LFS) was 40% and 51% for single and double unit recipients, respectively ($P = .35$) [41].

These results may have important scientific implications in terms of understanding the immunology of cord blood transplantation, the nature of the HSC niche and how modulation of this niche may impact upon transplant outcome.

## RIC Regimen Prior to Single or Double UCB Transplantation from Unrelated Donors in Adults

Most studies have tested UCB transplantation in the setting of myeloablative conditioning. An RIC regimen before UCB transplantation has been increasingly used to decrease toxicity, shorten the duration of aplasia, and extend the availability of CB transplantation to the elderly or patients who are not eligible for MA conditioning. The Minnesota group has evaluated the efficacy of UCB in the setting of a nonmyeloablative regimen consisting of Flu, Cy, and a single fraction of total body irradiation (TBI) (200 cGy) with cyclosporine (CsA) and MMF for posttransplantation immunoprophylaxis. The target cell dose for the UCB graft was $3.0 \times 10^7$ nucleated cells/kg, resulting in the selection of a second partially HLA-matched UCB unit in 85% of patients [24]. One hundred ten patients with hematologic diseases were enrolled. Neutrophil recovery was achieved in 92% at a median of 12 days. One cord blood unit predominated engraftment and none of the following factors were predictive of which unit eventually dominated: total nucleated, $CD34^+$, and $CD3^+$ cell doses; HLA matching; nucleated cell viability; ABO typing; sex match; or order of unit infusion. Treatment-related mortality (TRM) was 26% at 3 years. Survival and event-free survival (EFS) at 3 years were 45% and 38%, respectively.

More recently, the Société Française de Greffe de Moelle-Thérapie Cellulaire (SFGM-TC) in collaboration with Eurocord reported results of 155 consecutive UCB transplantations performed using an RIC regimen with a median follow-up of 18 months (range: 2-56) [42]. The median age was 47 years (18-69 years). Sixty-nine patients had myeloid and 22 lymphogenous acute leukemia (59%), 33 patients had other lymphoid malignancies, 18 had myelodysplastic syndrome (MDS), 8 myeloma, and 5 chronic myelogenous leukemia (CML). At time of transplantation, 20% of patients had active disease. Conditioning regimens consisted of Flu 150 to 200 mg/m$^2$, Cy 50 mg/kg and TBI 2 Gy. Two CBUs were infused in 59 (38%) patients. In the case of double UCB transplantation, the classification of HLA and ABO took into consideration the unit with the highest degree of disparity. Therefore, HLA identity was 6/6 in 5 patients, 5/6 in 28 patients, 4/6 in 93 and ≤3/6 in 8; 42 patients had a minor and 65 a major ABO incompatibility. The number of total nucleated cells and $CD34^+$ cells infused were $3.1 \times 10^7$/kg and $1.2 \times 10^5$/kg, respectively. The amounts were $2.8 \times 10^7$/kg and $1.4 \times 10^5$/kg for the single unit and $3.6 \times 10^7$/kg and $1.6 \times 10^5$/kg in double unit UCB transplantations, respectively. CsA and MMF were used for GVHD prophylaxis. Cumulative incidence of neutrophil engraftment at day +60 was 80% ± 3%, with a median time to achieve neutrophils >0.5/L of 20 days; autologous recovery was seen in 14% of the patients. In multivariate analysis, factors independently associated with better neutrophil recovery were CD34 cell dose (>$1.2 \times 10^5$/kg) (hazard ratio [HR] 1.51, $P = .04$), HLA compatibility (0-1 versus 2-3) (HR 1.5, $P = .05$) and previous autograft (HR 1.8, $P < .01$). Cumulative incidence of nonrelapse mortality (NRM) was 18% ± 3% at 18 months. The estimated probability of OS and DFS at 18 months was 62% ± 5% and 51% ± 4%, respectively.

In summary, both studies demonstrated the feasibility of RIC-UCB transplantation and reported encouraging results with this approach. Despite reducing the duration of aplasia, cumulative incidence of engraftment remains between 80% and 90%. Once again HLA disparity and cell dose played an important role and myeloid engraftment was achieved in 94% when patients received a well HLA-matched (6/6 or 5/6) CBUs with a higher CD34 cell dose (42).

### Use of Accessory Cells to Improve Engraftment

#### *Cotransplantation of an UCB unit with highly purified CD34$^+$ cells from haploidentical family donors*

Phase I-II clinical trials using accessory population(s) to enhance engraftment have been published, with interesting results. The Spanish group developed a strategy of UCB transplantation with coinfusion of a limited number of highly purified mobilized HSC (MHSC) from a human leukocyte antigen (HLA) unrestricted third party donor (TPD). Short posttransplant periods of neutropenia were generally observed in adults with hematologic disorders receiving UCBT with a relatively low cell content and 0-3 HLA mismatches after MA conditioning. This shortened neutropenic phase was was because of an early and initially predominant engraftment of the TPD-MHSC. After a variable period of double complete TPD + UCB chimerism, final full UCB chimerism was achieved (cumulative incidence >90%) within 100 days. Early recovery of the circulating neutrophils resulting from the "bridge transplant" of the TPD-MHSC reduced the incidence of serious neutropenia-related infections, also facilitating the use of drugs with myelosuppressive side effects to combat other infections. The observed incidence of GVHD and relapses was low, with OS and DFS curves comparable to those of HLA identical sibling transplants [27,28,43].

#### *Cotransplantation of an UCB unit with haploidentical parenteral multipotent mesenchymal stromal cells*

Mcmillan et al. [29] have reported an attempt to speed hematopoietic recovery in a single-institution phase I-II clinical trial in which ex vivo culture-expanded multipotent MSCs from haploidentical parental donors were infused at the time of UCB transplantation. Fifteen pediatric patients with high-risk acute leukemia were enrolled. Eight patients received MSCs on day 0, with 3 patients having a second dose infused on day 21. No serious adverse events were observed with any MSC infusion. All 8 evaluable patients achieved neutrophil engraftment at a median of 19 days. Probability of platelet engraftment was 75%, at a median of 53 days. With a median follow-up of 6.8 years, 5 patients remain alive and disease free. In a another pilot study [44] the Madrid group have used ex vivo-expanded BM MSC from parental donors that were infused at the time of the transplantation or the in case of refractory aGVHD. Nine patients received MSC immediately after CB and TPD highly purified mobilized HSC. Neither immediate adverse effects nor significant differences in CB engraftment or aGVHD development were observed. Four patients developed grade II aGVHD, refractory to steroids in 2. These reached complete remission (CR) after therapeutic infusions of MSC.

The results of both pilot studies show that infusion of ex vivo culture-expanded haploidentical MSCs into unrelated UCB transplantation recipients can be performed safely. Further studies may investigate the role of coinfusion of MSC to improve engraftment after UCB transplantation.

## CONCLUSION

Engraftment and other outcomes after UCBT are improving in the recent years, mainly because of better donor choices (cell dose and HLA matching), early patients referral for transplantation, improvement in supportive care, and greater center experience. Other approaches that improve engraftment after UCBT are being currently developed with very encouraging results. Those approaches may greatly increase the clinical use of cord blood cells for transplantation.

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